Microbial metabolism of ethylene

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The ethylene-oxidizing strain E20 was grown on different carbon sources to obtain information on the metabolism of ethylene from simultaneous adaptation studies and from measurements of specific activities of enzymes in cell-free extracts.

From the simultaneous adaptation studies it was concluded that ethylene oxide is a product of ethylene catabolism. The bacterium was also able to grow on the epoxide. From a comparison of the specific activities of isocitrate lyase and malate synthetase in different extracts it was concluded that the glyoxylate cycle was involved in the metabolism of ethylene, indicating that acetyl-CoA is a metabolite of ethylene catabolism. The sequence of reactions leading from ethylene oxide to acetyl-CoA could not be established from the simultaneous adaptation experiments and the enzyme activities in extracts.

INTRODUCTION

Microbial degradation of unsaturated aliphatic hydrocarbons has mainly been studied with organisms originally isolated on saturated alkanes. The initial oxidation step of 1-alkene metabolism by such organisms may occur by the same enzyme system either hydroxylating the methyl group or epoxidating the double bond. Further degradation products from the epoxide reported on several occasions include diols, α-hydroxy acids and saturated acids one carbon shorter than the substrate (van der Linden and Thysse, 1965; Klug and Markovetz, 1971). The physiological role of the epoxidation reaction in 1-alkane metabolism of microorganisms isolated on saturated alkanes is questionable. A Pseudomonas aeruginosa strain studied by Markovetz, Klug and Forney (1967) attacked the double bond of 1-alkenes but the bacterium failed to grow on tetradeca-1,13-diene and octa-1,7-diene. Abbott and Hou (1973) collected data for a Pseudomonas oleovorans strain suggesting that degradation of the epoxide arose via oxidation of the methyl group.
A functionally operating 1-alkene-degrading system has been reported by Watkinson (1973) who isolated bacteria growing at the expense of 1,3-butadiene. The catabolism of this compound presumably included butadiene monooxide and 3-butene-1,2-diol as intermediates. In this communication we report on the degradation route of ethylene which necessarily involves functional alkene breakdown when serving as sole carbon and energy source. Details on five strains of ethylene-utilizing bacteria have been recorded in the previous paper (de Bont, 1976).

MATERIALS AND METHODS

Medium. The composition of the mineral-salts solution (MS medium), used throughout the investigation, has been given previously (de Bont, 1976).

Chemicals. Acetyl-CoA, CoA, ATP and nicotinamide nucleotide coenzymes were obtained from SIGMA Chemical Company, Saint Louis. Ethylene (99.0%) and other gaseous substrates (commercial purity) were obtained from the Matheson Company, East Rutherford, N.J.

Organisms. Strain EO was isolated at 30°C by incubating 5 g of soil with 3 ml ethylene oxide in air in a 300-ml Erlenmeyer flask. After 4 weeks of incubation, 1 g of this soil was transferred to 10 ml MS medium in an 100-ml Erlenmeyer flask and upon sealing 2 ml of ethylene oxide was injected. Material taken from the slightly turbid incubation mixture after 3 weeks of stationary incubation was streaked onto plates of MS medium. Three large yellowish colonies had developed on these plates after 2 months of incubation in a desiccator vessel (3 litre) supplied with 2 ml of ethylene oxide. The pure culture was obtained after restreaking material of one of these colonies on plates of MS medium supplemented with 0.1% acetate.

Strains E 20, T1, T2, S and 32 were described previously (de Bont, 1976).

Growth of the organisms. Growth in liquid medium at 30°C while shaking occurred in 5-litre Erlenmeyer flasks containing 500 ml of the MS medium supplemented with the appropriate carbonaceous substrate (0.2% w/v). Gases (250 ml) were injected through a suba-seal mounted in the rubber stopper that sealed the flask. The inoculum consisted of organisms precultured on the same carbon source. The bacteria were harvested by centrifuging, washed with 0.03 M sodium phosphate buffer pH 6.8 and stored at -20°C in 10 mM Tris (hydroxymethyl)aminomethane buffer containing glutathione (2 mM).

Preparation of cell-free extracts. Cells were disrupted by ultrasonic disintegration (10 min) at 0°C. The extract was centrifuged and the supernatant fluid (2–6 mg protein/ml) used for assay.

Determination of protein. The Folin-Ciocalteu method was used (Herbert, Phipps and Strange, 1971).

Enzyme assays. Spectrophotometric estimations were conducted with a Gil-